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False-Negative Cerebrospinal Fluid Cryptococcal Latex Agglutination Tests for Patients with Culture-Positive Cryptococcal Meningitis

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Three cases of false-negative cerebrospinal fluid latex agglutination test results for patients with culture-positive cryptococcal meningitis are reported. False-negative results occurred in settings of low cryptococcal antigen concentrations in cerebrospinal fluid and were dependent on the latex agglutination test kit used. Investigation of each case revealed that prozone phenomena or interference from bound antibody or protein could not account for the false-negative results.

The latex agglutination test (LAT) for detection of cryptococcal capsular polysaccharide antigen in serum and cerebrospinal fluid (CSF) has been established as a reliable and rapid method for diagnosis of *Cryptococcus neoformans* infections (2, 3, 11, 19). Four commercial LAT kits, Crypto-LA (International Biological Laboratories, Cranbury, N.J.), MYCO-Immune (American Micro Scan, Mahwah, N.J.), IMMY (Immuno-mycologics, Norman, Okla.), and CALAS (Meridian Diagnostics Inc., Cincinnati, Ohio), have been widely available for more than 10 years and have been used extensively. Studies evaluating LAT kit performance with CSF samples have reported the sensitivities and specificities summarized in Table 1. However, there are well-described cases of false-positive LAT results obtained with CSF samples that have been attributed to interfering substances (1) and contamination with syneresis fluid (4, 15). False-negative CSF LAT results for patients with culture-positive cryptococcal meningitis have been reported to be rare (14) and are not as well characterized. One study reported false-negative CSF LAT results for all specimens from a single patient with culture-positive cryptococcal meningitis that resolved with dilution of the samples, suggesting a prozone phenomenon (20) (alternatively referred to as postzone [12, 21]). A second study noted a single false-negative CSF Crypto-LA LAT result that resolved with pronase treatment, even though this treatment is not normally recommended for CSF samples (13). This suggested the possibility that bound antibody or a nonspecific protein had masked the cryptococcal antigen in this sample (14). A third study, comparing three of the LAT kits (MYCO-Immune, CALAS, and IMMY), reported four false-negative CSF LAT results obtained with culture-positive samples and the IMMY kit that were positive by the two other kits (23). The false-negative IMMY CSF LAT results were attributed to less potent anticryptococcal globulin reagents supplied with the IMMY kit (23). A fourth study reported eight false-negative CSF LAT results for 88 AIDS patients with culture-confirmed cryptococcal meningitis (6). The investigators did not identify which LAT kit was used, but that report (6) suggested that the occurrence of false-negative CSF LAT results is more common than previously appreciated. This report describes false-negative CSF LAT results for three patients with culture-positive cryptococcal meningitis and the investigation of possible causes of this phenomenon.

Patient 1 was a 52-year-old man with a history of alcohol abuse who had a 1-month history of gait disturbance and 2 weeks of deteriorating mental status. He was afebrile and without meningismus. The patient had negative human immunodeficiency virus serologic results. Head computerized tomography scans showed mild atrophy. Lumbar puncture revealed CSF with 169 leukocytes (90% lymphocytes), glucose at 11 mg/dl, and a total protein concentration of 199 mg/dl. Intravenously administered antibiotics, amphotericin B, and oral antituberculous drugs were initiated as empiric treatment pending diagnostic tests. CSF was India ink negative, and a CSF CALAS LAT was negative for cryptococcal antigen. All bacterial cultures, including CSF, were negative. On hospitalization day 9, CSF cultures were positive for *C. neoformans*. At this point, portions of the patient's initial CSF sample were sent to two other hospitals, where they were found to be negative by the Crypto-LA LAT and positive by the IMMY and MYCO-Immune LATs at a titer of 1:4. Results did not change with dilution of the CSF samples or with pronase treatment. Despite amphotericin B therapy and addition of 5-flucytosine to the regimen, the patient expired 1 month into his hospital course. Four serial CSF samples taken during his hospital course were tested with the four LAT kits, and the results did not change (the results obtained with the Crypto-LA and CALAS kits were consistently negative, while those obtained with the IMMY and MYCO-Immune kits were consistently positive at titers of 1:4 and 1:2, respectively), independently of which technician performed the test or which kit lot was used.

Patient 2 was a 31-year-old man with AIDS and a prior history of opportunistic infections and renal insufficiency who had had a fever and a headache for 2 days. Head computerized tomography scans were significant for opaci-

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TABLE 1. Sensitivity and specificity of LAT kits for CSF samples

LAT kit	Sensitivity (%)	Specificity (%)	Reference(s)
Crypto-LA	98.6–100	96.7–100	7, 13, 14, 19, 22
MYCO-Immune	100	100	23
CALAS	100	100	13, 14, 22, 23
IMMY	82.6	100	23

TABLE 2. Summary of LAT performance with purified polysaccharide

Isolate no. or serotype	Final endpoint concn (ng/ml)			
	CALAS	Crypto-LA	IMMY	MYCO-Immune
1	2.8	2.8	5.5	2.8
2	1.7	3.5	3.5	3.5
3	0.9	0.9	0.9	0.9
A	5,[a] 7.6[b]	5,[a] 19[c]	0.63,[d] 157[c]	19[c]
D	10,[a] 61[b]	2.5[a]	2.5[d]	

[a] Reference 22.
[b] Reference 10.
[c] Reference 23.
[d] Reference 19a.

fication of the ethmoid and sphenoid sinuses. Lumbar puncture revealed CSF with seven leukocytes (90% lymphocytes), glucose at 76 mg/dl, and protein at 43 mg/dl. CSF was India ink negative, and a CSF CALAS LAT was negative for cryptococcal antigen. Intravenous antibiotic therapy was initiated. CSF cultures were negative for bacterial growth. The patient had a minimal response to therapy, and on hospitalization day 7 he experienced generalized tonic-clonic seizures. Subsequently, he developed hypotension and respiratory distress requiring incubation. Later that day, CSF cultures were positive for *C. neoformans* and intravenous administration of amphotericin B was initiated. The patient expired on the following day. CSF samples from the day of admission were sent to two other hospitals and found to be negative by the Crypto-LA and MYCO-Immune LATs and positive at a titer of 1:2 by the IMMY LAT. The CSF sample was then tested again with all four LAT kits at one hospital, and the same results were obtained regardless of which kit lot was used. Dilution of the CSF specimen or treatment with pronase did not alter the results.

Patient 3 was a 56-year-old man admitted for hematologic evaluation who was subsequently diagnosed with an early stage chronic lymphocytic leukemia. Results of serologic tests for human immunodeficiency virus were negative. Approximately 2 weeks into his hospitalization, the patient developed a low-grade fever, a headache, and photophobia. Head computerized tomography scans were unremarkable. Lumbar puncture revealed CSF which was India ink negative and positive for cryptococcal antigen at 1:64 with the MYCO-Immune LAT kit. CSF cultures were positive for *C. neoformans*. Amphotericin B therapy was initiated, and a repeat lumbar puncture was performed 3 weeks later. CSF LATs with the CALAS and Crypto-LA kits were negative, while LATs with the IMMY and MYCO-Immune kits were positive at a titer of 1:4. Dilution of the CSF sample or pronase treatment did not alter the LAT results, which were consistent regardless of which technician performed the test or which kit lot was used.

Isolates from each of the three patients were maintained on Sabouraud dextrose agar slants and were confirmed to be *C. neoformans* by the ability to hydrolyze urea, a positive bird seed agar response, the ability to grow at 37°C, and analysis with the API 20C system (Analytab Products, Plainview, N.Y.). Each isolate was shown to be *C. neoformans* var. *neoformans* by growth on CGB agar (17). India ink preparations revealed that each isolate had light-to-moderate encapsulation when grown in Sabouraud dextrose broth at 30°C. Genomic DNA was extracted from each isolate and examined by Southern blot analysis of *URA5* gene restriction fragment length polymorphisms, as previously described (5), to investigate the possibility that all three isolates were genetically related. *Hae*II digestion revealed a different *URA5* restriction fragment length polymorphism pattern for each strain (data not shown). Capsular exopolysaccharide was purified from the culture supernatant of each isolate as previously described (16), and its concentration was determined by the phenol-sulfuric acid method (8). The purified polysaccharide was serially diluted and tested with all four LAT kits in accordance with the package insert instructions for CSF samples until endpoints for detection were reached. The results are summarized in Table 2.

Three cases of culture-positive cryptococcal meningitis with false-negative CSF LAT results that occurred in situations with low cryptococcal antigen concentrations in CSF are described. Detection of cryptococcal antigen was dependent on which LAT kit was used. Only the IMMY kit gave positive results for all of the CSF samples from all of the patients. The Crypto-LA and CALAS kits gave consistently negative results for all CSF specimens in which cryptococcal antigen concentrations in CSF were low. The MYCO-Immune kit gave positive results for CSF samples from two of three patients in this setting. None of these false-negative results resolved with dilution of the specimens or with pronase treatment. A similar phenomenon was noted in a study comparing the performance of three LAT kits (IMMY, MYCO-Immune, and Crypto-LA) in which four false-negative CSF LAT results were reported for the IMMY kit but identified as positive with the other two kits (23). Interestingly, this occurred in a setting of low cryptococcal antigen concentrations in CSF (positive Crypto-LA CSF LAT reported at titers of 1:2 to 1:16 for these specimens). In contrast to the present report, the pattern of false-negative CSF LAT results by kit was different in that study, with the IMMY kit being the least sensitive test.

The initial investigation focused on the characterization of the three *C. neoformans* isolates. Since the cases described here came from two hospitals in a limited geographic area, it was possible that a single strain was responsible for the phenomena observed. Restriction fragment length polymorphism analysis showed that each isolate was genetically distinct. It has been suggested that false-negative LAT results could be due to "capsule-deficient" *C. neoformans* strains (7). This is not the case here, since each isolate had the capacity to produce capsular polysaccharide in vitro and in vivo. A yield of 50 to 60 mg of polysaccharide was recovered from the culture supernatant of each isolate after 10 days of growth in 50 ml of Sabouraud dextrose broth.

Since LAT titer results obtained with kits from different manufacturers can vary considerably with the same sample (14, 23), it is possible that the false-negative CSF LAT

results reported here reflect different sensitivities of the kits. In a setting of low antigen concentrations in CSF, less sensitive LATs may give false-negative results because the antigen concentration is simply below the level of detection of the kit. The data presented in Table 2 argue against this explanation. When purified polysaccharide from each isolate was tested with all four kits, there was not more than a onefold dilution difference in the endpoints observed between kits. While there was some variation between isolates in the limits of detection of capsular polysaccharide by the four kits, there was little or no variation in the limits of detection of any given isolate when tested with all four kits. Furthermore, the evidence suggests that the capsular antigen produced by all of these strains is highly reactive with the anticryptococcal reagents in all four LAT kits. The final endpoint concentrations in Table 2 are either lower than or consistent with the lower-range values of previously reported limits of detection for each kit (Table 2). However, the possibility exists that in vivo capsular polysaccharide production could result in qualitative differences in antigen expression that could account for the differences observed in this study.

Treatment with pronase should eliminate the possibility that bound antibody or a nonspecific protein differentially interfered with the reagent of one kit and not with that of another. However, the possibility still exists that a nonprotein interfering substance was present in the CSF and preferentially reacted with the reagent of one kit but not with that of another. Thus, at low concentrations of cryptococcal antigen in CSF, such interference may result in a pattern of false negatives with the kits that are subject to increased interference.

False-negative CSF LAT findings can result in delayed diagnosis and therapy for patients with cryptococcal meningitis. While it is clearly stated in the package inserts of all four kits that a negative LAT result does not exclude the diagnosis of cryptococcal infection, particularly when only a single specimen is tested and the patient exhibits symptoms consistent with cryptococcal infection, this is probably not appreciated by many physicians. This report will further serve to alert physicians and laboratory personnel to the fact that in settings of low cryptococcal antigen concentrations in CSF, a false-negative CSF LAT result may be more likely to occur and may be dependent on which LAT kit is used. Low antigen concentrations in CSF are more likely to be associated with non-AIDS cases of cryptococcal meningitis than with those of patients with AIDS (18). However, it should be noted that low cryptococcal antigen concentrations in CSF can occur in a significant proportion of AIDS patients, as evidenced by the experience of patient 2 and reports in the literature (6, 9). In evaluating patients with symptoms consistent with cryptococcal infection and negative CSF LAT results, retesting of the CSF with alternative LAT kits may resolve the diagnostic dilemma and avoid treatment delay. We do not recommend that laboratories switch from one LAT kit to another for routine evaluation of specimens on the basis of the data presented here. We suggest that evaluation of currently available LAT kits, as well as evaluation of new technologies for detection of cryptococcal antigen (such as monoclonal antibody-based LAT kits and enzyme-linked immunoassay systems), include careful evaluation of samples with suspected low antigen concentrations. It is also strongly recommended that for every CSF sample for which a diagnostic LAT is ordered, fungal (and routine) cultures be automatically obtained as well. In addition, CSF fungal cultures should be carefully inspected daily for at least 1 week so that detection of initial growth is not delayed.

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